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Bioprospecting of Endophytic Plethora from *Ocimum sanctum* for Antifungal Activity

Seemadevi Suresh Kadam, *Shivangi Shivraj Kanase
P.G. Department of Microbiology, Yashvantrao Chavan Institute of Science, Satara,415 001, Maharashtra, India.
*Corresponding author: srkanase@rediffmail.com

ABSTRACT
During the last couple of decades, occurrence of superficial and invasive mycoses among human population, especially involving immunocompromised patients, has been intensely increased. Continuing development of resistance amongst fungal pathogens to antifungal drugs, side effects associated with the use of current antifungal drugs and many other limitations associated with existing antifungal drugs has led various streams of sciences to have an increased interest in searching novel antifungal compounds having low toxicity and high efficiency. 'Traditionally medicinal plants have been exploited as an excellent source for extraction of therapeutic agents including antifungal agents. However, due to over exploitation; many medicinal plants are on verge of extinction in near future. The study of endophytes associated with medicinal plants might offer the most excellent possible way of obtaining novel metabolites with potential antifungal activity without diminishing plant diversity. So the aim of this study was to investigate antifungal potential of endophytes of Ocimum sanctum. Cross streak and agar overlay methods were followed for preliminary antifungal screening. Among the 70 fungal and 33 bacterial endophytes isolated, only one bacterial isolate, showed anti-Aspergillus activity but failed to show anti-Candida activity. The results suggested that healthy leaves can be a potential source for the isolation of endophytes with anti-Aspergillus activity. The ethyl acetate extract prepared from the fermentation broth exhibited better inhibition against Aspergillus species. In conclusion, exploring the potential niches like the endosphere could open the vast area of opportunity for investigating newer antifungal drug leads.

Keywords: *Ocimum sanctum; anti-Aspergillus activity; Endophytes; Drug Leads ; Ketoconazole.*

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INTRODUCTION

Recent reports on mucormycosis and other fungal infections in post covid cases, frequent emergence of new diseases, drug resistance in microbes, and remarkable increase in occurrences of fungal infections among human population are some of severe public health concerns all over the globe. Currently one of a constant and serious threat to health of humans is invasive mycoses caused by *Aspergillu, Candida, Cryptococcus,* and *Pneumocystis species.* Approximately 1.5 million deaths all over the world every year are owing to these fungal infections [1,2]. There are only restricted number of antifungal drugs available in market, that too with shortcomings associated with them such as toxicity, safety, spectrum of activity, as well as pharmacokinetic properties [2]. As compared to antibacterial drug development, search of novel antifungal drug is much challenging since fungi are one of the eukaryotic cells and many probable targets for antifungal therapy are also observed to be existing in human beings with significant host toxicity risk [3,4]. Further, out of four approved classes of antifungals like polyenes, echinocandins, azoles, as well as fluoropyrimidine analogs; two i.e. polyenes and azoles, target same components of fungal plasma membrane [5]. Thus limited number of cellular targets upsurges chances for fungi to develop resistance to available antifungals.

Considering current status of infection by different fungi, the present study was focused on antagonism against *Aspergillus species* and *Candida species*. Both immunocompetent and immunocompromised patients are susceptible to wide spectrum of clinical infections caused by these two pathogens. Occurrences of postoperative *Aspergillus* infections comprising endocarditis, graft infection as well as osteomyelitis, has been frequently reported [6,7] . *A. fumigatus* is mainly responsible for around 90% of *Aspergillus* induced disorders in humans, but the infections by other species like *A. flavus,* A*. niger, A. terreus and A. nidulans* have been also reported [8]. Presently, invasive aspergillosis is most frequent source of infectious pneumonic mortality in the patients having heart, lung, pancreas, renal and bone

marrow transplants [9,10].Research objectives concerning novel antifungal agents highlighted some major points like, newly discovered drug must be potent enough at levels which are readily achievable at infection sites and must possess negligible or no host toxicity. Considering these aspects, it is necessary to focus unexplored ecological niches as promising sources of natural bioactives which are eco-friendly, renewable and easily obtainable [11].Traditionally medicinal plants have been exploited as an excellent source for extraction of therapeutic agents however, due to over exploitation of medicinal plants along with other biotic interferences, many medicinal plants have now listed as critically endangered moreover they are on verge of extinction in near future. Further plants, as compared to most drug producing microbes, exploited by the pharmaceutical industries, are often expensive and are not easy to propagate on commercial scale because of seasonal specificity, restricted availability of land for their cultivation and environmental competence etc. The extensive extraction procedures for valuable and potent bioactive compounds will have the risk of depletion of valuable medicinal plants diversity. Moreover, national along with international regulations restrict transport of many native plant species [12]. However, if endophytes can secrete similar uncommon and significant bioactive agents like host plant, this would not only diminish the necessity of harvesting slow growing rare plants, but also will aid to conserve continually diminishing biodiversity of the globe. In recent years microbial endophytes have emerged as a promising substitute for plant secondary metabolites with potent antifungal properties. Endophytes are microbes that reside within healthy living tissues of their host plants without causing any harm to them. [13].They are proved to be producing many secondary metabolites which are antibacterial, antiviral, antifungal, antioxidant, antidiabetic, anticancerous, insecticidal, immunosupressive, antimalarial and cholesterol reducers etc. and may perhaps be promising source of antifungal substances. Many secondary metabolites of endophytes have proved to be promising drug leads. Above facts provided the rationale to investigate the antifungal activity of compounds produced by endophytes, especially those isolated from medicinal plant *Ocimum sanctum* which is extensively distributed in the Indian environment. It has been used traditionally in treatment of several diseases including microbial infections and is widely applied in herbal therapy. In Ayurveda *O.sanctum* has been used from long back ago for its various healing properties, renowned as general stimulant and vitalizer, 'The Elixir of Life'. Leaves of this have been reported for getting relief from common cold, cough , bronchitis, and digestive disorders [14]. Leaves were shown to contain volatile oil that shows antifungal, antibacterial, antiasthmatic, antisuppressant, analgesic properties [15,16]. Leaves of *Ocimum* have been found to be effective against aflatoxin producing fungus *Aspergillus parasiticus* [17]. While much is known about the phytochemistry of *Ocimum sanctum*, its endophytes have not been fully explored for medicinal values. So aim of present study was to explore endophytes for antifungal potential with objectives of isolation, screening and evaluation of antifungal activity against *Aspergillus species* and *Candida species.*

MATERIAL AND METHODS

All chemicals used in present work were of analytical reagent (AR) grade and were purchased from Sigma Aldrich Chemicals Company, unless otherwise specified. Fungal and bacterial culture media were procured from Himedia, Mumbai.

Site and collection of plant samples:

Plant samples targeted for study were collected from relatively pollution free site at medicinal plant garden of Satara College of Pharmacy, Satara, (Approx: 17°41'29.04"N,74° 0' 3.37" E) in Maharashtra state, India. Apparently healthy, symptomless, disease free and mature plants were selected during monsoon as per need .Leaves were cut off with an ethanol-disinfected sickle and placed in pre-sterilized zip lock plastic bags. The collected plant samples were processed within 8 hours for isolation of endophytes .

Isolation of endophytes:

The collected plant samples were initially washed with running tap water for 7-10 mins. followed by sterile distilled water before their surface sterilization. Surface sterilization was carried out by using method of Petrini (1991) [18]with some modification considering the fact that the time required in each sterilent is very much crucial and usually varies depending upon the host plant, tissue thickness, texture and sensitivity. Washed leaves were initially soaked for 4 min. in 70% ethanol followed by rinsing with sterile distilled water. These samples were then immersed in 5% Sodium hypochlorite with 5 % available chlorine for 6 min and then rinsed thoroughly with sterilized distilled water. Then were immersed in 70% ethanol for 1min. Finally all were thoroughly rinsed three times with sterilized distilled water for three times. Excess water was allowed to surface-dry on sterilized filter paper under laminar airflow chamber. Then, smaller pieces of size approximately 1cm X 1cm were positioned on the sterile Nutrient Agar medium (NA) with Ketoconazole (100µg/ml) whereas for fungal endophytes, sterile Potato Dextrose Agar (PDA) medium supplemented with chloramphenicol (50 µg/ml) was used. Plates were incubated at 30

±2°C for 3-5 days and at 27±2°C for 2 to 3 weeks for bacterial and fungal endophytes respectively. Pure cultures obtained were preserved at low temperature in refrigerator. Confirmation of efficiency of surface sterilization process was done by inoculating 0.1 ml from final rinse on respective culture media as control plate [19,20]. The success of sterilization protocol was confirmed by observing no microbial growth onto culture media in control plate.

Primary screening of endophytes for antifungal activity:

Microorganisms used for antifungal activity:

Two fungal cultures used in current investigation, *Aspergillus species (*MCC 1074) *Candida albicans* (MCC 1154) were procured from National Centre for Microbial Resource of National Centre for Cell Science (NCCS) Pune. Since, a large number of isolates have to be evaluated for the antifungal activity; preliminary screening was done using different screening methods.

Preliminary anti-*Candida* activity by bacterial endophytes:

Preliminary anti-*Candida* activity was performed by making use of cross streak method. Wherein, sterilized Nutrient Agar (NA) plates were firstly inoculated with overnight grown cultures of endophytic bacteria as a single streak at the centre and subsequently incubated at 35 ± 2°C for 3-5 days. Then overnight grown culture of test organism, i.e. *Candida albicans* (MCC1154) was streaked at right angle to producer bacterial endophytes and monitored for inhibition of growth after 24-48 hours.. The experiment was performed in triplicates as well as repeated twice. The endophytic isolates that have shown inhibition against the test organisms after incubation of 24 hours were recorded as positive and the endophytic isolate with the maximum inhibition potential was carefully chosen for further studies. Whereas isolates that hasn't inhibited the test organisms were recorded as negative and were not investigated further.

Preliminary Anti-*Aspergillus* activity by bacterial endophytes:

All isolated bacterial endophytic strains were spot inoculated separately at centre of sterilized NA plates and were incubated at 35 ± 2°C for 72 hours.Full grown endophytic colonies were then exposed to chloroform for 20 mins for inactivation. This was followed by aseptic over layering of 100 microlitre of standard spore suspension of *Aspergillus species* (10^6- 10^7spores / ml) mixed with sterilized 15 ml of semisolid PDA. These plates were subsequently incubated at 27± 2°C for 3-4 days and were monitored for inhibition halos.

Preliminary anti-*Candida* activity by fungal endophytes: Anti-*Candida* activity of isolated fungal endophytes was tested by agar plug diffusion method [21] with some modifications. 100 µl of standardized test suspension of *Candida albicans* (MCC 1154), was spread evenly on sterilized PDA plates using sterile glass spreader. Endophytic fungal isolates were cultured at ambient temperature for 7-14 days on antibiotic free Potato Dextrose Agar. Actively growing endophytic fungal agar blocks having 6 mm diameter were positioned on surface of PDA plate inoculated with test organism using sterile cork borer. Agar block without endophytic fungal growth and antibiotic were used as negative control. These plates were kept at 4 °C for 4 h for allowing diffusion of antifungal metabolites, and were subsequently incubated at 37 °C for 24 h. and were observed for zone of inhibition around the block.

Preliminary anti-*Aspergillus* activity by fungal endophytes:

To check anti-*Aspergillus* activity of fungal endophytes, dual culture method was used wherein both fungal endophytes and test pathogenic fungus i.e*. Aspergillus species* were cultured separately on PDA plates at 25±2°C for 5 days .Grown fungal mycelium was cut by using cork borer with inner diameter 6 mm. and both the fungal discs were placed on opposite sides of same PDA plate at 5 cm distance apart from each other. Whereas control is prepared by placing the same sized fungal disc of test pathogen i.e. *Aspergillus species* only (without endophytic fungi) on PDA plate. All plates were incubated at 25±2°C for 5 to 7 days and then were observed for antagonism at the point of interaction [22].

Lab scale production and extraction of antifungal compounds:

Bacterial endophyte that shows promising antifungal activity in preliminary screening were selected and were cultured in 50 ml presterilized nutrient broth at ambient temperature for 24 h on orbital shaker with 150 rpm speed in order to obtain active culture. This active culture was inoculated and grown in 500 ml capacity Erlenmeyer flask containing 300 ml. sterilized nutrient broth and further incubated at ambient temperature for 72 h on orbital shaker with speed of 150 rpm. After incubation culture broth was subjected to centrifugation at 10,000 g for 20 mins at 4°C and the cell free supernatant was collected. This was then extracted with ethyl acetate. During extraction cell free supernatant was added with the equal volume of ethyl acetate in a separating funnel and then vigorous shaking was carried out for 10-15 mins. This was followed by keeping it steady for 5-10 mins. for separation of layers. The upper layer of organic solvent containing antifungal metabolites was then separated and this extraction step was repeated for three times. This extract was subjected to evaporation of organic solvent using rotary vacuum evaporator and crude extract thus obtained was stored at 4° C until further studies.

Secondary antifungal assay:

The crude ethyl acetate extract obtained in above step was analyzed for activity against *Aspergillus species* (MCC 1074), by making use of agar well diffusion method. For anti-*Aspergillus* activity, test fungus was initially grown on PDA until a sufficient spore formation has reached. 0.85 % sterile saline solution was added onto fungal growth and spores were released by means of a sterile swab by gently scrapping surface of colonies. Spore suspension was removed by using micropipette. Density was adjusted to $1X10^5$ spores/ ml concentration. McFarland Standards was used as reference for adjusting required density of microbial suspensions so as to obtain their number within a specified range. The above suspension of test fungi were then aseptically mixed homogenously in a 15 ml sterilized PDA. It was then over layered on sterile PDA basal agar and allowed to solidify. By means of sterile cork borer having 8 mm diameter, wells were prepared and filled with 100 microlitre of crude ethyl acetate extract in DMSO. To control wells, corresponding aliquot of pure DMSO and standard antifungal drug ketoconazole were added to prepare negative as well as positive controls respectively. Then plates were placed in refrigerator at 4 ^{o} C for 4 h. to allow thorough diffusion of antifungal compounds and subsequently incubated at 28±2^{0}C for 3-4 days. After incubation, zone of inhibition was measured around each well. All antifungal assays were performed twice in triplicates. The antifungal activities are indicated with the average of the values determined ± standard deviation (SD). While means were compared by means of one sample t test ($p \leq 0.05$) .The statistical analysis of the data was performed using open source online statistical software, Quickcalcs-Graphpad software.

RESULTS

Isolation of endophytes:

Optimization of surface sterilization protocol was a significant prerequisite for the isolation of plant endophytes. The result of efficacy of surface sterilization protocol was determined. For ensuring the isolation of exclusively endophytic microbes, surface sterilization of plant material was carried out along with sterility checks i.e. last water rinse from the plant samples were inoculated on respective culture media. Observations of any fungal and bacterial growth in sterility check was the indication that the surface sterilization method was improper and in such cases, the obtained isolate has to be discarded. In this way surface sterilization protocol was optimized. The surface sterilization protocol mentioned above was found to be highly effective wherein sterility checks showed no growth, which claimed the successful surface sterilization procedure, with no epiphytic contamination and maximum endophytic recovery. Thus, the isolates obtained in the present investigation were true endophytes.

Isolation of endophytes from *Ocimum sanctum* leaves was done as per method of Petrini (1991) [18]. After incubation fungal growth was obtained on 9th day and bacterial growth on 3rd day around the leaf explants along the periphery .Many of the endophytic isolates showed similar morphological features. After proper examination through visible differences, including colony characteristics like colony colour (white, green, grey, brown, yellow, orange, off-white, black etc.), Colony texture (velvety, cottony, smooth, wrinkled etc.), colony size, shape ,growth pattern (Fast, moderate, slow growing), Colony elevation (raised, flat), colony margin, pigmentation and production of exudates , the endophytes showing absolute resemblance were considered as repeats. Thus, only those isolates were carefully chosen for the further investigation which showed variation in morphological features and thereby they were considered as different endophytic isolates. As a result of this a total of one hundred and three endophytes were isolated from approximately 254 tissue segments of selected plant. Thus obtained 70 fungal and 33 bacterial endophytes were then subcultured on fresh sterile NA and PDA plates and were suitably coded as Osa NA L1 to Osa NA L33 for bacterial isolates and, Osa SA L1 to Osa SA L 70 for fungal isolates.

Preservation of culture

Stock cultures of bacterial and fungal endophytes were maintained by subculturing on NA and PDA respectively at monthly intervals. After growth at p^{H}7 and temperature 25-30^{o}C for 2-7 days, the slants were maintained at 4-8^{o}C. From actively growing stock cultures, subculturing was done on fresh media. This was used as the starting material for fermentation experiment designed for obtaining antifungal compounds.

Preliminary screening of endophytes for antifungal activity:

Preliminary anti-*Candida* and anti-*Aspergillus* activity by bacterial endophytes:

Out of 33 bacterial endophytes used for screening, only one (3%) isolate designated as Osa NA L1, showed anti-*Aspergillus* activity against test microorganisms in agar overlay method but failed to show anti-*Candida* activity.

Preliminary anti-*Candida* and anti-*Aspergillus* activity by fungal endophytes:

The fungal endophytes were examined for antifungal antagonism against *Aspergillus species* as well as *Candida albicans*. It was surprising to observe that none of fungal endophytic isolates exhibited significant anti-*Candida* and anti-*Aspergillus* activity. Whereas most of the commercially used antibiotics are fungal products. However negative results do not mean that bioactive constituents are absent or these endophytic fungi are not of any potential applications, it may contain other active chemical components that might produce a definite other physiological action.

Lab scale production and extraction of antifungal compounds:

The bacterial endophyte Osa NA L1 from *Ocimum sanctum* leaves, which exhibited significant anti-*Aspergillus* activity during preliminary screening, was selected for this step to optimize fermentation period and temperature, so as to enhance the qualitative and quantitative yield of antifungal metabolites. Initially the endophytic bacterial isolate Osa NA L 1 was checked for its ability to produce metabolites at different culture conditions. Fermentation of this isolate resulted in luxuriant growth in the selected nutrient media. Endophytic bacteria from *O. sanctum* leaves shown characteristics turbidity, with pellicle formation. The metabolites produced by these isolate was maximum when was grown in nutrient broth at 34°C and pH 7.0 for 03 days. Further extraction of antifungal metabolites was carried out by using ethyl acetate. After evaporation of organic solvent, solid crude extract was suspended in 5 % dimethylsulphoxide (DMSO) and was used as crude organic solvent extract. This extract was evaluated for secondary antifungal activity against, *Aspergillus species* (MCC 1074).

Secondary antifungal assay:

After mass production of antifungal metabolites by *Ocimum sanctum* endophyte (Osa NA L 1), the confirmation of antifungal activity was done using crude extract against *Aspergillus species* (MCC 1074) by means of agar well diffusion method. To assess the magnitude of antifungal action, the metabolite was co-assayed with standard reference antifungal agent i.e. pure ketoconazole. All the clear zones of inhibition observed were measured in terms of millimetres and results were tabulated as diameters of zones of inhibition accordingly in Table 1. Crude ethyl acetate extract of Osa NA L1 has shown anti-*Aspergillus* activity with diameter of zone of inhibition as 11 mm. The control i.e. pure DMSO had no inhibitory activity and a significant zone of inhibition was observed for Ketoconazole.

Table 1: Zones of inhibition in mm against selected fungal pathogens ± SD – standard deviation; – No inhibition.

Bacterial Endophyte from/ Control	Crude extract	Zone of inhibition in mm against	
		Candida albicans	Aspergillus sp.
Ocimum sanctum	Osa NA L 1	-	11.33±0.52
Negative control	Pure DMSO	-	-
Positive control	Ketoconazole (1000µg/ml)	29.33±0.52	33.00±0.89

DISCUSSION

Therapy against systemic fungal infections is still a big task in health care. A novel antifungal to be approved for clinical use typically requires a long period of 8 - 10 years. In order to increase efficacy of existing antifungals, efforts are necessary so as to reduce toxicity, enhance bioavailability, improve its spectrum of activity and combat resistance. Even though synthetic antifungal drugs contribute to a major percentage in use, natural antifungals have their own place in anti-mycotic market. Hence our study is aimed to identify antagonistic endophytes of medicinal plant against *Candida* and *Aspergillus*. From 254 explant pieces of *Ocimum sanctum* leaves inoculated, only 103 endophytes, comprising 70 fungal and 33 bacterial endophytes were recovered. The failure of other endophytes from explants may be due to inability to complete their life cycle outside the environment of their original host [23]. Additionally, interactions between an endophyte and its host are mutual exploitation and both organisms need each other to survive [24]. Saikkonen *et al.* (2004) [24] further explained that endophytic lifestyle is complex and adaptation to the environment involves multi-species interactions as well as biotic and abiotic influences. Without appropriate environmental conditions, some endophytes will eventually die even though all nutrients necessary for growth are supplied. In this study, only the culturable endophytes were isolated and assemblages do not represent non-culturable endophytes. In this study, leaves were used for isolation of endophytes since in traditional medicine, these were reported to exhibit diverse bioactive potential including antifungal, antibacterial in addition to insecticidal [25]. Yu et al. (2010) [26] suggested to use healthy leaves, showing no disease symptoms and cultivated at pesticide free atmosphere but surrounded by infected plants, are more likely to be selected. The fungal endophytes were most prevalent in leaves and this may perhaps due to thin cuticle layers [27]. Tong et al. (2011) [28] also

revealed that endophytes penetrates leaves of the host plant most prevalently. Besides, a larger surface area (4 cm in length and 3 cm in width), a thin cuticle layer of leaves might provide better opportunities to fungal endophytes for penetration and colonization. It is also assumed that leaf being plant photosynthetic area may induce more density of endophytes. Tong et al. (2014) [29] proposed crucial necessity of optimization of surface sterilization protocol to ensure success of endophytic isolation because a short immersion time might not be sufficient to remove all epiphytes whereas a extended immersion time might cause considerable damage to delicate samples such as leaf which further affect the viability of endophytes residing in host plant sample [30]. Oyebanji et al. (2009) [31] stated use of sodium hypochlorite is enough to remove the epiphytic microbes, dirt as well as debris on leaf samples and these protocols are most frequent choice for surface sterilization in most laboratories. Furthermore, maturity of host plants, and environmental factors for instance rainfall and atmospheric humidity may perhaps affect the diversity of the endophytes [32]. Antimicrobial potential of endophytes from medicinal plants has been reported by many workers. Verma *et al.* (2009) [33] reported antibacterial antagonism of endophytic actinomycetes from *Azadirachta indica* against *E. coli*. Jalgaonwala *et al.* (2010) [34]reported antibacterial potential of bacterial endophytes from roots of *Aloe vera*. Roy and Banerjee (2010)[35] isolated and determined antimicrobial activity of endophytic bacteria from *Vinca rosea* against *Bacillus cereus, Escherichia coli* and *Klebsiella pneumoniae.* Pal *et al.* (2012) [36] reported antagonism of bacterial endophytes of *Passiflora foetida* against *Staphylococcus aureus, Escherichia coli,* and *Klebsiella pneumoniae*.

Various reports regarding isolation of fungal endophytes and their antimicrobial activity by others, similar to our studies are available [37–40]. Various antifungal metabolites have been reported from endophytic fungi belonging to diverse structural classes like peptides, alkaloids, terpenoids, steroids, quinines, phenols, and flavonoids [41]. Similarly leaves of *Ocimum sanctum* has been found to be efficient in inhibition of alfatoxin producing fungi *Aspergillus parasiticus* [17]. Crude ethyl acetate extracts of *Macrophomina phaseolina* (OSHL-2.1) exhibited significant inhibition against *F. oxysporum, B. cinerea* and *R. solani* [25].Tiwari et al. (2010) [42] obtained four endophytes from *Ocimum* exhibiting inhibition of five fungal phytopathogens and found variations in its ability to inhibit all five fungi. Chowdhary and Kaushik (2015) [25]reported antifungal antagonism of about 23% of the fungal endophytes isolated from *Ocimum sanctum.*As against this in our study no fungal endophyte has shown antagonism against *Aspergillus species* and *Candida species.*

This may be perhaps due to the reason that endophytic fungi require compounds produced by their host plant so as to promote their growth and enhance production of antifungal agents. Furthermore, choice of culture media is also vital in obtaining antifungal compounds from endophytes. A number of researchers have studied the relationship between host plants and associated endophytes, and shown that addition of host plant extract can improve the growth of endophytes as well as enhance antimicrobial production [43]. This state is due to a long period of relationship between them that has established connection through continuum of mutualism where some compounds produced by the host are necessary for the endophytes. Consequently, it is crucial to understand such relationships and this knowledge may be well exploited and applied for production of more superior drugs from these endophytes. Endophytic bacteria are found in virtually every plant on earth. Various parts of plant like stem, root and nodule [44] , stems,leaves, as well as root [45] may also be used as a source material to isolate endophytic bacteria. Uptil date a much work is carried out on endophytic fungi but scanty information is available on endophytic bacteria with antifungal potential. Endophytic bacteria have been reported to be antagonistic against phytopathogens [46–49]. Our study reported antifungal endophytic bacterium from *Ocimum sanctum* leaves against *Aspergillus species* which has not been earlier reported. Few reports are available on phytopathogenic and antibacterial antagonism of bacterial endophytes from *Ocimum sanctum*. Tiwari, (2010)[42],isolated four endophytic bacteria OS-9, OS-10, OS-11, and OS-12 from asymptomatic leaves of *Ocimum sanctum*. These were assessed against various phytopathogenic fungi viz. *Sclerotium rolfsii, Fusarium solani, Alternaria solani, Rhizoctonia solani,* and *Colletotrichum lindemuthianum* by dual culture method. Out of all, strain OS-9 was reported to be antagonistic to *Rhizoctonia solani, Alternaria solani, Fuusarium. solani,* and *Colletotrichum lindemuthianum* whereas OS-11 was shown to be antagonistic against *Alternaria solani*. Singh, A. K., & Kumar Sharma, R. (2018) [50] isolated endophytic bacterial strains from *Ocimum sanctum* leaves, and investigated their antibacterial potential against Gram positive bacteria, *Staphylococcus aureus*, *Streptococcus pyogens* ,*Bacillus cereus* and Gram negative bacteria, *Escherichia coli*, *Salmonella typhimurium* and *Klebsiella pneumoniae.*

To our knowledge, present work illustrates first time isolation of an endophytic bacterial strain from' *Ocimum sanctum leaves* with anti-*Aspergillus* potential. Further studies to identify this endophyte and evaluate antifungal potential of purified compounds as well as their mode of action are worth for its commercial application.

CONCLUSION

This study shows that *Ocimum sanctum* harbor diverse types of endophytes and one bacterial endophyte exhibited significant inhibitory activity against pathogenic fungus *Aspergillus species.* Further investigations regarding identification of this endophyte and isolation, purification along with characterization of these antifungal compounds are crucial as an approach to investigate novel natural antifungal drug leads which may help to widen the library of potential antifungal agents against *Aspergillus species* for pharmaceutical applications. It can be concluded that bacterial endophytes harbored in leaves of *Ocimum sanctum* hold great promise not only as natural antifungal drug lead against pathogenic *Aspergillus species* but also as pharmaceutical preservative in different formulations, in cosmetics, in food-preservation and as an antifungal component in wall paints etc.

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CONFLICTS OF INTEREST/COMPETING INTERESTS:

The authors declare that they have no conflict of interest.

AUTHORS CONTRIBUTIONS

Shivangi Shivraj Kanase conceived and designed the experiments and involved in reviewing, and editing the manuscript.Seemadevi Suresh Kadam conducted experiments and wrote the manuscript. Both authors read and approved the final manuscript.

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This article is original and not published elsewhere. Both authors discussed the results, read and approved the final manuscript. The authors confirm that there are no ethical issues in the publication of the manuscript.

ETHICS OF HUMAN AND ANIMAL EXPERIMENTATION: NA

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